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PEST MANAGEMENT WITH INSECT SEX ATTRACTANTS
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EPA's Registration Requirements For Insect Behavior Controlling Chemicals—Philosophy and Mandates

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For those of you who do not know, I regretfully report that Dr. Len Axelrod suffered a fatal heart attack a few weeks ago, and needless to say, I feel very humble standing here in his place.

During the few years in which I had the distinct pleasure of associating with Len, he always received my deepest respect, not only as a scientist, but as a humanitarian who was greatly concerned with the welfare and survival of man and his environment.

So, without any further adieu, I would like to dedicate this presentation to Dr. Leonard R. Axelrod.

During the past year, Criteria and Evaluation Division has worked closely on a contract with the Zoecon Corporation. The purpose of this contract was to provide scientific and technical data to enable the Environmental Protection Agency to develop guidelines for the registration of pheromones and insect growth regulators. Part of what I am about to present is from the Zoecon Contract, as we take our first major step in developing the necessary guidance and criteria for the development and registration of chemicals for insect behavior control.

Since we are just beginning to develop these guidelines, I am not going to discuss the specifics on what needs to be done for evaluating efficacy, environmental chemistry, safety, etc. However, I will attempt to address the past, present, and future in our association with these materials for use not only as alternatives for some of the more highly persistent traditional pesticides, but also as an aid in reducing the chemical load entering the environment.

Historical Background

Although European naturalists as early as the 17th Century utilized caged virgin female moths to lure males for their collections, the field of chemical communication did not acquire appreciable sophistication until quite recently. In 1959,

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Karlson and Luscher coined the term "Pheromone" to refer to substances which are emitted to the outside by an individual and received by a second individual of the same species which responds in a specific manner, such as a behavioral response. (The singular term "Pheromone" refers to one or several chemical compounds eliciting the response). Two years later, the German chemist Butenandt and co-workers culminated a 20-year effort by identifying the male sex attractant from 500,000 silkworm moth females, the first such natural chemical message system to be decoded. These two events plus an increased interest in alternate pest control methods have helped stimulate numerous research programs directed toward the understanding of the biological function of the chemical structure of pheromones.

For the purposes of the EPA Guidelines for Registration, the term "Pheromones" will also include non-pheromone chemicals, which elicit or modify behavioral responses. Included in this category will be attractants discovered by empirical screening.

A Sampler of Behavioral Functions

To date pheromones have been found to mediate a diverse repertoire of behavior patterns ranging from those in unicellular organisms to those in the primates. As a partial sampler, insect pheromones have been found to regulate such phenomena as aggregation responses, including trail following as in ants and termites, host colonization as in attraction or mass attack of bark beetles to trees, and recruitment ("Calling") of sexual partner as in attraction of males to female moths. In other chemical stimuli systems, dispersion away form the released pheromone occurs. This occurs in the case of an alarm substance emitted from an attacked aphid which causes the aphid's neighbors to escape predation by rapidly dropping from the plant. Alarm pheromones can also serve to mark an enemy for attack by additional individuals, a phenomenon many of us have encountered after being stung by a social hymenopteran such as a bee or a wasp. Other patterns of pheromone response include colonial behavior in many social *Hymenoptera* (such as bees and ants) and the *Isoptera* (termites). Generally, the queen emits the "master" pheromone which serves many functions including colony membership identification and eliciting the retinue behavior of feeding and grooming the queen. Examples of some of the chemicals that mediate behavior are listed in Table I. More extensive information can be found in recent reviews by Jacobson and Shorey.

The sensitivity of the messenger reception system to the pheromone is of an extraordinary nature. In the male silkworm moth, a single pheromone molecule can elicit firing of an olfactory neuron on the male antenna, whereas some 200 molecules are required for an overt behavioral response as male movement toward the odor source.

Table I: Examples of Chemicals Mediating Various Behavioral Responses in Insects*

Scientific Name	Common Name	Chemical Name	Response
Limonius californicus	Sugar Beet Wireworm Beetle	Valeric Acid	Male Attraction
Porthetria dispar	Gypsy Moth	cis-7,8-Epoxy-2-Methyloctadecane	Male Attraction
Trichoplusia ni	Cabbage Looper Moth	(Z)-7-Dodecen-1-ol Acetate	Male Attraction
Anthonomus grandis	Boll Weevil	1) (+)cis-2-Isopropenyl-1-Methylcyclobutanethanol 2) (Z)-3,3-Dimethylclohexylideneethanol 3) (Z)-3,3-Dimethylcyclohexyli-Deneacetaldehyde 4) (E)-3,3-Dimethylcylohexylideneacetaldehyde	Female & Male Attraction (Aggregation)
Apis mellifera	Honeybee	1) 2-Heptanone 2) Isopentyl Acetate	Alarm
Zootermopsis nevadensis	Termite	Hexanoic Acid	Trail-marking

*References can be located in Jacobson, M., In Rockstein, The Physiology of Insecta, 2nd ED., 3:229-276. 1974 (Academic Press, New York)

Elucidation of the chemical structures of pheromones has lagged behind the description of the general behavioral functions. Generally, evidence of the presence of a pheromone by observation of the specific behavior must precede bioassay and isolation. In 1966, several insect pheromones were chemically identified, and since then the number of compounds characterized has increased steadily, due in part to the increased sophistication of the instrumentation for chemical analysis. As the synthetic pheromones have become available, biologists have been able to decipher their exact behavioral function and applied entomologists and zoologists have been able to initiate programs designed to utilize pheromones for pest population manipulation.

Principles

Generally, the development of pheromones into a chemical system for pest population manipulation, such as mass trapping or disruption of pheromone communication, initally involves chemical characterization of the pheromone system, chemical synthesis, and laboratory and field documentation of the behavioral responses elicited by the pheromone. Once the structure and evoked biological response are described, evidence of the effectiveness and usefulness of the pheromone for population manipulation must be established through field testing under natural conditions.

Use of Pheromones in Pest Monitoring

The most immediate and obvious use of synthesized pheromones has been for population monitoring, essentially an extension of the early naturalists' use of female moths to lure males. A synthetic pheromone in a sticky trap, for example, lures and ensnares the pests. The numbers caught can be used to estimate the pest's relative abundance and seasonal development. This information may permit decisions to be made as to the exact timing of insecticide applications for maximum effectiveness or even a determination of the necessity for a conventional pesticide application. For certain crops, this precise population sampling technique may allow substantial reductions in the standard pesticide application, with the use of only 0.015 to 0.00003 gram of synthetic pheromone per acre in a single trap.

A typical case of such a benefit occurs with the monitoring of the summer fruit tortrix moth in the Netherlands. The maximum benefit for an insecticide spray with this species is derived when the application occurs a few days after egg hatch, just as the larvae are migrating to their feeding sites. This susceptibility window can be predicted accurately through the use of pheromone traps, and their widespread use in the Netherlands has resulted in the use of fewer insecticide applications with more effective control.

Similar monitoring programs for a number of pests such as the codling moth, the peach twig borer, and the oriental fruit moth are in the research and development stage. Population monitoring with commercially available pheromone traps has been adopted by some commercial growers as part of integrated pest management programs. Monitoring systems are not regulated by the EPA since this pheromone use is neither designed nor claimed to suppress population levels.

Mass Trapping for Population Suppression and Control

Several distinct methodologies for pheromone utilization in direct population manipulation have been described. The technique of mass trapping utilizes compounds which lure one or both sexes to a mating and/or aggregation locus. In mass trapping these compounds are emitted from a dispenser and the attracted pest becomes ensnared in a sticky trap or a device of similar principle. Success of this method depends upon removal of a sufficient portion of the pest population so as to reduce damage or nuisance to an economically or medically acceptable level.

Efficacy in mass trapping involves not just the description of the actual number of pests captured in a test, but, additionally, it requires some independent estimate of the effect on the total pest population and/or of the damage reduction.

In some successful mass trapping experiments, the quantity of pheromone used per acre was less than 0.04 grams (0.00009 lbs) or roughly 1/10,000 the quantity per acre for several conventional pesticides. In the case of other pest species, the total amount of pheromone per acre could be substantially less than this amount.

In mass trapping an attractant-baited trap generally serves to capture the pest. Traps are set out at a density which is sufficient enough to remove the proportion of the breeding pest population necessary for population suppression.

For example, in the case of the redbanded leafroller moth, a species in which the female-produced pheromone lures the male for mating, 40 attractant traps per acre in an apple orchard will remove enough males to effect control of this pest, providing that this technique is used on low initial populations. For this species, a low population could be achieved by application of a conventional insecticide prior to the initiation of the pheromone program. A low population also would exist in a well-managed commercial apple orchard.

A mass trapping population manipulation technique would possess the following advantages over conventional pesticides:

1. Highly specific control of the target organism;
2. Facilitation of biological control of non-target pests;
3. Use of a compound which is designed to attract rather than kill at rates comparable to natural pheromone levels.

Population Suppression by Disruption of Communication

Another promising means of using pheromones or attractants in an integrated pest management program is through disruption of pheromone communication, a procedure first demonstrated by Gaston and co-workers. Population manipulation involving disruption of communication is dependent upon atmospheric permeation with a sufficient concentration of communication component chemicals to prevent or greatly alter the pest's olfactory perception and orientation toward the natural pheromone sources.

Research experiments with many moth species have demonstrated that emission of these communication disruptants into the air prevents the male's successful location of the female, thereby effecting a reduction or elimination of mating. The disruptant employed could be the natural pheromone chemicals reconstituted in a different proportion or rate, or a compound which by itself is not part of the natural communication system but which affects the behavioral response. Successful communication disruption thus generally prevents crop damage in the following generation by reduction or elimination of mating in the present generation. Efficacy evaluation again requires precise sampling of total pest populations and accurate estimates of crop damage.

One technique for dispersal of the pheromone into the atmosphere is emanation of the compound from point source dispensers. Such evaporating devices can be deployed at a set number per unit area and are recoverable (i.e., can be removed after use), so that the pheromone is dispersed only into the atmosphere and not sprayed directly onto the foliage, crops, soil and target or non-target organisms.

Another method of pheromone application is similar to that used for conventional pesticides. In this procedure, a pheromone formulation (involving microencapsulation, wettable powders, or other volatile release matrices) is sprayed with standard pesticide equipment.

Experiments conducted to date indicate that rates of only 10 grams (0.02 lbs) or less of pheromone per acre per application may be more than adequate for communication disruption for several weeks. The specific test protocol necessary to demonstrate efficacy will be dependent on the characteristics of the pheromone compound, the nature of the manipulation technique chosen, the nature of the crop, the timing of the application, and the biology of the organism.

Current EPA Philosphy

The public's concern for the environment is one of the factors motivating the development of new agents for insect population control. At the present time, most research, notably in industry is still concentrated on hard chemical insecticides,

especially directed toward modifications of existing structural types such as organophosphates, organotins and carbamates. The emphasis is on agents with less persistence, improved efficacy, and greater specificity to target organisms.

Insect pheromones in general are highly selective; each pheromone is limited to a particular insect, or at most, to several related species. The most efficient pheromones appear to be chemicals which are produced by the insects themselves, or by host plants. They are effective in extremely small quantities. They are attractive to a particular stage of the pest insect, generally the adult stage, and they are used only during a limited time of the year. By their very nature, natural pheromones have been presumed to be relatively non-toxic to man and other non-target organisms. However, extensive investigations concerning the possible hazards or adverse effects of pheromones to man and the environment are necessary to determine the safety of their use as pesticides, especially when synthesized.

Although pheromones exhibit highly desirable characteristics as insect control agents, commercial interest in chemicals which are effective against a single species on a sporadic need basis is generally limited. Understandably, the chemical industry does not wish to bear the high cost of research and development for materials with limited profit, however, the USDA is carrying out the development of pheromones as part of their thrust toward integrated pest management systems.

At the present time pheromones are mainly used as survey tools for monitoring populations and many investigators feel that this use of pheromones will be the key to integrated pest control programs for many crops. Pheromone baited traps for monitoring purposes have the following specific uses:

1. To determine proper timing of sprays;
2. To locate sources of infestation from over-wintering areas;
3. To detect a new pest coming into a region;
4. To determine if a pesticide spray is actually needed.

In the future, pheromones may be used for control of certain insects. Current thinking is that control with pheromones may be effective in two ways.

1. By mass trapping of one or both sexes;
2. By confusion of the insects through communication disruption, i.e., the insects become confused and die before mating occurs.

Certain of the compounds are included in major programs being carried out as part of the thrust toward integrated pest management, in particular, gossyplure for pink bollworm, grandlure for boll weevil and disparlure for gypsy moth.

Pheromones for numerous insects are available for experimental purposes and a small number are available for commercial uses. Because of the long experience of industry and government with chemical pesticides, the registration requirements and

development procedures are best defined for the "traditional" chemical control agents. Safety, efficacy and environmental requirements which have evolved for traditional insecticides are the standards by which practically every new chemical control agent is evaluated by the EPA. The cost of developing any new and novel method of insect control, falling within the jurisdiction of the EPA, is going to be at least as expensive as the cost of developing a traditional chemical insecticide. In addition to the baseline requirements established for the traditional insecticides, new agents (even in the "experimental permits" and "temporary tolerance" phases), may be required to undergo additional studies some of which may be new and unique.

Proposed Regulations and Recommendations

Safety-Hazard Evaluation. Hazard evaluation requirements should strongly consider the use pattern and the basic safety of the individual materials. The requirements may be entirely different if the pheromone is applied by point-source release versus being broadcast over an area in the form of a spray or granular formulation. Also, with a pheromone, one must consider whether or not the compound occurs in nature, and possibly in what concentrations. A further distinction in the requirements might also be made between a point-source release mechanism which is not recoverable versus a point-source release mechanism which is recoverable.

Efficacy - Use Pattern Criteria - Labeling. With pheromones it is felt that some term should be used other than "control". There is a wide variation of terms that could be used, but, generally, it is felt that terms such as "population suppression" or "aids in control" are more suitable particularly where the usefulness and efficacy of the product are based on its ability to protect foliage and/or on its ability to prevent mating, thus leading to possible population reduction in a manner other than by direct kill as experienced with traditional hard chemicals. Perhaps the most controversial area in obtaining pheromone registration revolves aroung the fact that even though there may be a reduction in mating this does not necessarily mean there is a reduction in the population of a species. This is why the term "aids in control" may be more appropriate for pheromones which are employed as an integral part of an integrated pest management system. In addition, resistance to pheromones, especially those used for monitoring, should not develop, thus insuring a longer market life for pheromones.

Catches in pheromone baited or organism-baited traps or mating incidence in the treated areas are supportive of efficacy but such data do not constitute a demonstration of efficacy.

Field Plot Techniques. Field plot techniques acceptable for traditional pest control in general are acceptable for pheromones but may vary depending upon the biology of the insect and

adaptability of the product to certain pest management practices. Each individual pheromone development must be considered on a case-by-case basis, and criteria such as plot size, number of replicates, geographical test locations, etc., should, out of necessity, be subject to revision depending upon the problem at hand.

Areas which may have to be investigated in addition to what is presently done with traditional pesticides are as follows:

(A) Necessity for obtaining more details on the biology of the insect, the stage of development present at time of application and the percentage of the population in each stage.
(B) The necessity for compiling past history records on insect populations, on the treated area and in adjacent areas.
(C) The necessity for compiling records on adjacent crops and areas, for the season of the experiment and, possibly, for the seasons before and after treatment.

Current Registration Status

When used as survey tools in integrated pest management programs, the pheromones are not subject to the Federal Insecticide, Fungicide and Rodenticide Act, unless used in conjunction with a pesticide which is subject to the Act. If used as an attractant in a control procedure or in insect mitigation, pheromones are subject to regulation under the Act.

The pheromone field as a part of the science of entomology has advanced rapidly in the past decade, and in particular, in the past few years. At present, muscalure is registered as an attractant in fly baits. This pheromone is specific to houseflies, for which the baits are so intended; however, other species of flies may be attracted by other ingredients in the bait. The rationale for registering muscalure differed from other basic attractants in that its action as a sex attractant incorporated in the bait, permitted a reduction in the concentration of a more toxic chemical pesticide and increased the efficacy of the product. This constituted the first registration of a pheromone for insect control.

Extensive field experiments have been conducted by the USDA to assess the potential of disparlure in controlling the gypsy moth by communication disruption techniques. Due to the method used and type of results obtained, it will be necessary to employ criteria for registration based on foliage protection and/or inability of the insect to mate and propagate the species, rather than on the basis of population reduction obtained through direct kill. Disparlure is in the advanced stages of development under an experimental permit, and registration appears possible within a year or two.

Experimental permits have also been issued by EPA for codlemone, a sex attractant for codling moth, and grandlure, the

boll weevil aggregation pheromone, which is currently in for registration.

Current Progress

Scientists in the Registration and Criteria and Evaluation Divisions have begun a joint effort to develop guidance and criteria for the efficacy and safety evaluation of pheromones. The purpose of this guidance will be to assist registrants with the registration requirements for developing and testing these compounds, and to assist the review process with the evaluation of such products when submitted for registration.

The "Guidance and Criteria" package will be developed in a separate document paralleling the general registration guidelines in format, but applicable specifically to the development and testing of these type compounds.

This will further enable us to circulate and work on this document with the Registration Division until the requirements and methods are suitable to both Criteria and Evaluation Division and the registration process, thereby preventing the problem of constantly modifying the general registration guidelines. The anticipated completion date for the pheromone guidance and criteria will be the end of the current calendar year, as we move forward in the area of new pesticide products.

PB 278 104

A Root Isolation Method for Testing Root-Active Chemicals[1]

S. L. DUFFY[2]

Abstract. A method for isolating roots of a growing plant was devised. The technique allows treatment of an isolated portion of the root system without disturbance or injury to the untreated root mass. A specially designed planter-box was used to induce natural separation of the root system in 3 to 4 weeks. Weeping willow (*Salix babylonica* L.) seedlings were used to test the performance of dichlobenil (2,6-dichlorobenzonitrile), paraquat (1,1'-dimethyl-4,4'-bipyridinium ion), sodium hydroxide (NaOH), and copper sulfate ($CuSO_4 \cdot 5H_2O$) in reducing the viability of treated roots. A tetrazolium chloride assay was used to determine the difference in viability of the treated roots. Copper sulfate and paraquat-treated plants produced symptoms of systemic injury.

INTRODUCTION

Roots of trees and other ornamental plants often grow through cracks and connecting joints in sewer and drain lines. These roots frequently block and sometimes completely stop liquid flow. As a result, mechanical or chemical removal of these roots is necessary. Mechanical removal of roots from sewer and drain lines is temporary (1). Therefore, root-killing chemicals are used to control root penetration, growth and regeneration in sanitary sewer and drain systems.

Various methods for testing root-active chemicals have been reported (1, 4, 5, 6, 7). However, more comprehensive methods are needed that can be successfully used in confirmatory laboratory testing of root-active chemicals. Consequently, our laboratory has developed a root isolation method that subjects the roots of the test plants to an environment more closely related to field conditions without the loss of flexibility and rapidity which are highly desirable for laboratory testing.

MATERIALS and METHODS

Four-month-old weeping willow seedlings were used as test plants. At the time of testing, the main stem of the plants was approximately 0.7 to 1.0 cm in diameter, and the plants were 30 to 50 cm tall.

The growth box used in this method was a modification of the box designed by Muzik and Whitworth (8) (Figure 1). The frame of the box was constructed from 1.5 cm exterior plywood. The box was stained with redwood paint immediately after construction. No wood preservatives were used because of possible phytotoxic effects. There were no apparent phytotoxic effects from the redwood stain. The growth box can be used several years if it is stained annually. The dimensions of the box used in this method were 37.5 by 32.5 by 27.5 cm (Figure 2). Plexiglass was used as side panels. These panels were 30.7 by 30.7 cm and were fitted into a groove that was slanted 60° from the base of the box. Approximately 120 small holes, 3 to 4 mm in diameter were bored across the front plexiglass panel 8 cm from the base. Metal shutters 30.5 by 28 cm were made to fit the ends of the box to prevent the entrance of light. A sand trap was constructed by placing a smaller metal shutter, 30.5 by 7.7 cm, and a piece of exterior plywood, 29 by 11.5 by 1.2 cm, at the base of the box (Figure 3). This trap was filled with masonry sand to within 10 mm of the top. The willow seedling was planted in the box so that the root mass was adjacent to the holes in the plexiglass. The plants were allowed to grow in the greenhouse at 23 to 26 C with a photoperiod of 15 hr. The plants were watered daily with a garden hose equipped with a sprinkler head. Both foliage and soil surface were thoroughly wetted. A moisture differential was maintained during the period the sand trap was in place. The sand in the sand trap was maintained at near field capacity by watering several times daily. Whereas the soil in the growth box was kept at a low moisture level. After 3 to 4 weeks, the roots of the willow seedlings had grown through the holes in the plexiglass into the sand trap. The sand trap was then removed and the sand carefully washed from the roots. The test roots were selected and the remaining roots removed. A glass dish 20 by 10.5 by 8 cm served as the container for the liquid nutrient medium into which the test roots were submerged. Hewitt's medium (3) was used as the nutrient solution. The test roots were submerged in 320 ml of half-strength Hewitt's medium for 14 days before the herbicide was added (Figure 4).

Copper sulfate, sodium hydroxide, dichlobenil, and paraquat were applied to the roots in half-strength Hewitt's medium at the rate of 8.45 g/L. The roots were exposed to the test herbicides for 24 hr. Roots submerged in only half-strength Hewitt's medium served as controls. After 24 hr, the roots were removed from the medium, washed 2 hr in continuously running tap water, blotted dry, and weighed. The roots remaining in the growth box were also washed in tap water, blotted dry, and weighed. The difference between the fresh weight of the roots that were submerged in the liquid medium and those remaining in the growth box was used as the basis for calculating the percent of root system exposed to the test herbicide. A 250-mg sample of root tissue was removed from the exposed root portion of all test plants and tested by the tetrazolium chloride assay method (4).

RESULTS and DISCUSSION

All herbicides satisfactorily reduced the viability of the exposed roots (Table 1). Some color interference can be expected from

[1] Received for publication April 28, 1975.

[2] Plant Physiol, U.S. Envir. Prot. Agency, Agr. Res. Ctr.-E, Bldg. 402, Beltsville, MD 20705.

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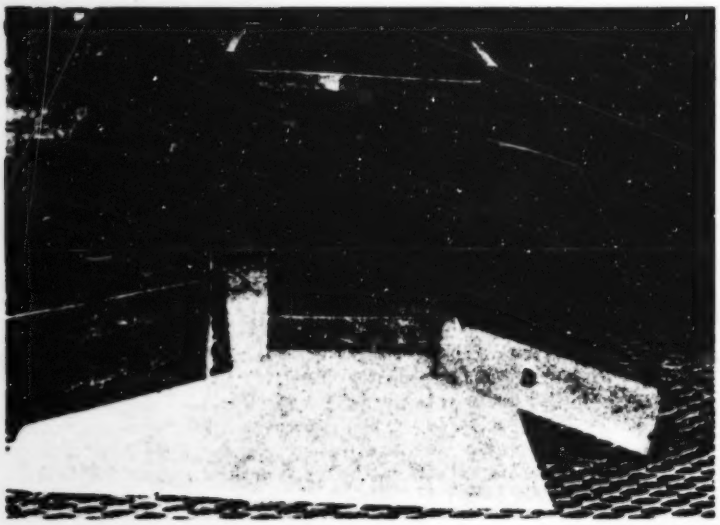

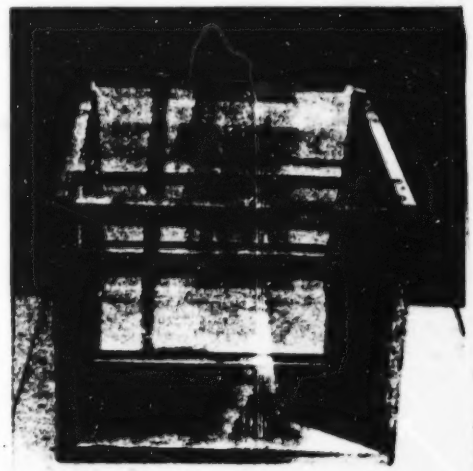

Figure 1 (top, left). Disassembled growth box showing: A. Box shell; B. front plexiglass panels (note holes for root induction); C. shutter to prevent entrance of light; D. sand trap retainer; E. sand trap wall; and F. back plexiglass panel.

Figure 2 (top, right). Assembled growth box. Note holes in bottom for drainage.

Figure 3 (bottom, left). Willow seedling during root induction period. Note sand trap assembly.

Figure 4 (bottom, right). Willow seedling after root induction period showing exposed roots submerged in half-strength Hewitt's medium.

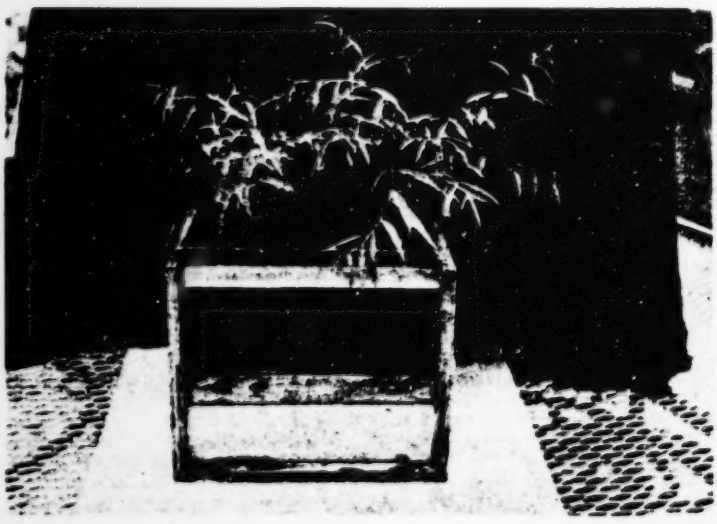

Table 1. The response of willow seedlings to root-active chemicals 24 hours after treatment.

Treatment (concn 8.45 g/L)	Portion of root system treated (%)	Decrease in root viability[ab] (%)	Phytotoxicity ratings (aerial parts)[ac]
Copper sulfate	32	91 b	3.0 b
Dichlobenil	46	70 b	1.0 a
Paraquat	34	77 b	4.1 c
Sodium Hydroxide	45	73 b	1.0 a
Untreated Control	25	0 a	1.0 a

[a]Means followed by the same letter are not significantly different at the 5% level as determined by the Duncan multiple range test.

[b]Percent decrease in viability = $\frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control}} \times 100$

[c]Phytotoxicity ratings: 1 = no apparent injury; 2 = very slight injury; 3 = moderate injury; 4 = severe injury; 5 = death of plant.

the sodium hydroxide treatment due to the red coloration produced by the hydrolytic action of the sodium hydroxide on the root tissue. The washing technique used in the split-root method (4) was modified by subjecting the roots to continuously running water. This was done in an effort to reduce this interference. Copper sulfate and paraquat-treated plants produced symptoms of systemic injury 24 hr after treatment (Table 1). Systemic injury from copper sulfate treatments has been reported by Ahrens (1) and Duffy (4). Systemic injury appears to be related to the mobility characteristics of the herbicide, the concentration of the herbicide, and the percentage of the root system exposed to the herbicide. Other conditions such as transpiration rate, abundance of foliage and kind of foliage may be contributing factors to systemic injury (5). Further study is needed to determine the importance of each of these factors in producing systemic injury.

The tetrazolium chloride assay used in this method has been described by Duffy (4). However, in this test, no boiled root standard was used and the test tubes were shaken mechanically for 2 min instead of manually. The optical density (OD) of the ethanol extract was read at 520 nm. The tetrazolium chloride assay for viability has been used in the past in herbicide research. Burt et al., used the assay to test the viability of johnsongrass [*Sorghum halepense* (L.) Pers.] roots (2). Purcell and Young (9) and Stephonkus and Lamphear (10) used the assay to study viability of plant tissue following cold injury. Roots of comparable age and size show more consistency in the color reaction than do roots of various ages and sizes.

The root isolation method can be adapted easily for radioisotope, plant growth regulator, and plant nutrition studies. Root and top growth of test plants can be controlled by pruning. Therefore, stock plants can be maintained indefinitely for testing.

The root isolation method has been tested in our laboratory 8 times over a period of 2 yr. It has proved to be very reliable.

ACKNOWLEDGMENTS

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Decay of Parathion Residues on Field-Treated Tobacco, South Carolina—1972 (II)

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ABSTRACT

In an effort to confirm the results of a study in 1971 to determine the length of time required for parathion to degrade to "zero" levels, parathion was applied twice at a rate of .375 lb/acre to field tobacco in South Carolina. After each application, parathion degraded to "zero" levels in 5 days. These results tended to confirm the findings of the original study in which the maximum time required for parathion to degrade to zero levels was estimated to be 7 days and the minimum time 2 days. Weather was characterized by scanty rainfall and temperatures averaging 76° F. During the original study, rainfall was heavy and daily temperatures averaged 80.9° F.

Introduction

A study reported by Keil *et al.* (*1*) was carried out in June-July 1971 to observe the decline with time of levels of parathion normally applied to field tobacco. This paper reports the results of a second study conducted in June-July 1972 to confirm the findings of the 1971 study.

Methods and Procedures

All growing, application, sampling, and analytical techniques were identical to those of the earlier study (*1*). Cokers 319 variety tobacco was planted on April 17, 1972. Plots consisting of three 12-foot rows were randomly selected for treatment with parathion, endosulfan, parathion in combination with endosulfan, or as appropriate controls. Each treatment or control plot was replicated four times in a completely randomized design for a total of 16 plots. Guard rows were used to reduce pesticide drift. In the earlier study, parathion and endosulfan were applied as sprays at rates of .375 and .125 lb active ingredient (A.I.) per acre, respectively, on June 8, 21, and July 8, 1971. In the present study, parathion and endosulfan were sprayed at these same rates on both June 12 and 28, 1972.

Results and Discussion

Results of analyses for parathion residues are given in Table 1 and indicate that parathion residues decayed to "zero" levels in 5 days after each application. Practical "zero" was assumed when statistical differences between actual zero (none detected) and observed values did not exceed the calculated LSD (least significant difference at the .05 level). The results tended to confirm the findings of the original study in 1971 in which the maximum time required for parathion to degrade to zero levels was estimated to be 7 days and the minimum time 2 days.

Endosulfan residues were not measured, but tobacco from the endosulfan-treated plots was analyzed for parathion to determine if there was a measurable amount of drift between plots. The parathion-endosulfan treatment was included to determine if any decay interaction existed between the two chemicals. This was noted at one sampling period, 1 day after application, i.e., more parathion residue was present when parathion-endosulfan was applied. This effect was noted in the previous study up to and including the third day after application.

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Weather during the 1972 study was characterized by scanty rainfall and temperatures averaging 76° F (Table 2). In the 1971 study, rain had been in excess of 12 inches and daily temperatures averaged 80.9° F. Results of both studies support the findings of Maier-Bode (*2*) who concluded that rainfall does not significantly affect residue levels of parathion.

See Appendix for the chemical names of compounds discussed in this paper.

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(*1*) *Keil, J. E., C. B. Loadholt, B. L. Brown, S. H. Sandifer, and W. R. Sitterly. 1972.* Decay of parathion and endosulfan residues on field-treated tobacco, South Carolina—1971. Pestic. Monit. J. 6(1):73-75, Erratum (This issue).

(*2*) *Maier-Bode, H. 1970.* Parathion residues. Dtsch. Med. Wochenschr. 95(48):2457.

TABLE 1.—*Parathion residues on field-treated tobacco by treatment plots, South Carolina—1972*

Application Date	Sampling Time in Days from Last Application	Mean Parathion Residue Level in PPM on Four Replicate Treatment Plots			
		Parathion—Treated Plot (.375 A.I. lb/acre)	Endosulfan—Treated Plot (.125 A.I. lb/acre)	Parathion (.375 A.I. lb/acre) / Endosulfan (.125 A.I. lb/acre)—Treated Plot	Control Plot
June 12	0	0	0	0	0
	1	1.30	.21	3.37	.05
	3	.46	.05	.26	.03
	5	.03	.01	.09	0
	10	0	0	0	0
	15	0	0	0	0
June 28	1	1.98	.29	1.54	.05
	3	.48	.02	.13	.02
	5	.07	0	0	0
	9	0	0	0	0
	15	0	0	0	0

NOTE: $LSD_{.05}$ = least significant difference at 95% probability level = .50 and may be applied across time or residues.

TABLE 2.—*Temperature and rainfall during parathion residue degradation*

Period	Time to "Zero" Residue [1]	Temperature (°F) Lows	Highs	Avg. [2]	Rainfall (inches)
June 12-17	5 days	50-67	80-87	72	0
June 28-July 3	5 days	68-70	87-93	80	(July 1) 1.08

[1] "Zero" residue level was assumed when statistical differences between actual zero (none detected) and observed values did not exceed the calculated LSD (least significant difference at the .05 level).
[2] Overall average temperature, 76° F.

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Field Study of the Safety of Abate for Treating Potable Water and Observations on the Effectiveness of a Control Programme involving both Abate and Malathion*

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Abate is a larvicide for Aedes aegypti, *a vector of yellow fever, dengue, and haemorrhagic fever. It is less toxic than malathion or DDT. Tests in human volunteers had previously indicated it would be safe to add to drinking-water where the mosquito often breeds.*

In a 19-month test in Puerto Rico, Abate was added to the drums and cisterns in which a community of about 2000 persons stored their drinking-water. This use of Abate and other insecticidal measures (malathion spraying) gradually achieved control of A. aegypti *during the first 4 months of treatment and control was maintained thereafter although the test area was surrounded by active breeding-sites. There was no accumulation of Abate in the water in spite of monthly additions of the compound. With minor exceptions, residents readily accepted this use of Abate. Careful surveillance failed to reveal any illness or significant side-effects attributable to measures adopted in the control programme. Abate is considered safe for full-scale field use according to directions.*

The organic phosphorus compound, Abate (*O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate) (OMS-786), produced neither clinical ill-effects nor detectable changes in blood cholinesterase levels when given under controlled conditions to volunteers at a dosage of 64 mg/man/day for 30 days (Laws et al., 1967). In view of these findings and of the results of toxicity experiments in animals (Gaines, Kimbrough & Laws, 1967), it was considered safe to undertake a limited field trial in an area where larvicidal treatment of open barrels of stored drinking-water was necessary to achieve effective mosquito control.

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It has been demonstrated that treatment of such stores of drinking-water with Abate sand granules at the nominal rate of 1 ppm is highly effective for controlling the larvae of *Aedes aegypti* (Brooks, Schoof & Smith, 1965). It has also been shown that not all of the Abate added to water is mobilized. The compound hydrolyses slowly so that the highest concentration of Abate reached, following a single application at the recommended rate, is about 0.5 ppm. Even when drums of water were re-treated at 3- and 6-week intervals at a nominal rate of 1 ppm, the Abate concentrations in the water did not increase appreciably above 0.5 ppm (Brooks et al., 1967). Thus, the maximum dosage received by people who drink water at the usual rate of 2 litres per day is about 1 mg/man/day if the water is treated with Abate according to directions.

Because Abate had not been used previously for control of mosquitos in drinking-water, it was considered a good idea to maintain medical as well

as entomological surveillance during its introduction for this purpose. Through the active participation of official health agencies, an attempt was made to learn of any unusual illness among the hundreds of participants in the study. In addition, more detailed attention was given to a small number of volunteers, who were willing to give periodically all the samples of blood and urine the laboratory was prepared to analyse. Chemical studies of these samples involved the known effects of Abate, and the observers were especially alert for any clinical effects characteristic of organic phosphorus compounds even though no effect of this or any other kind was expected.

METHOD

The necessary clearance for the study was obtained from the Puerto Rico Department of Health. An area called Cerro Gandia, located on a high hill, in the town of Manatí, Puerto Rico, was selected as the site of the field trial. During the first 16 months of the study, the area was served by 5 exterior public faucets. However, the water pressure was intermittent and frequently low, and, as a result, much of the drinking-water was either rainwater stored in cisterns or water transported from lower ground and stored in open drums or barrels outside the dwellings. The study area comprised the equivalent of about 9 square blocks. It had 401 premises and a population of approximately 2000 persons. At the beginning of the study, drinking-water was stored in 190 metal drums, 27 wooden barrels, and 10 cisterns. During the 16th month of the study, a 4-inch (10-cm) water main was connected to the existing water line, and one faucet was added so that there were then 6 faucets and enough water pressure to make the supply effective. Considering the placement of the faucets, the new system was capable of servicing conveniently about two-thirds of the study area. Just prior to the study, the index of *Aedes aegypti* infestation in outdoor containers in the 9-block area was found to be positive in 41% of the premises.

Local health authorities and public health nurses were briefed on the protocol of the field trial and on the symptoms and treatment of organic phosphorus poisoning. Residents of Cerro Gandia were told they were free to refuse treatment of their water if they wished.

Initially, 20 volunteers were requested for the donation of blood and urine samples. They received no rewards and were free to discontinue participation at any time. The volunteers, who were obtained without difficulty, were screened for a history of illness. The results are summarized in Table 1.

TABLE 1

POSITIVE FINDINGS ON MEDICAL SCREENING OF 38 VOLUNTEERS PRIOR TO LARVICIDAL TREATMENT

Finding	No. of cases
Allergy to detergents	1
Arthritis	1
Asthma	1
Congestive heart failure	1
Frequent headaches	3
Hypertension	4
History of kidney disease	3
Night sweats	1
Pregnancy	4
Tuberculosis under treatment	1
Varicose veins	1

No volunteer was rejected on the basis of medical history. Because the group studied earlier was composed of adult males only (Laws et al., 1967) the Manatí group was purposely selected to include females primarily and to cover a broader range of ages. The volunteer group eventually totalled 37 women and 1 man, with ages ranging from 15 to 83 years. The average age was 36; the median age, 31. Four of the women were known to be pregnant. The distribution by age-group was as follows: 15–20 years, 6 women; 21–30 years, 8 women; 31–40 years, 9 women; 41–50 years, 4 women; 51 years or more, 10 women and 1 man. Turnover in the volunteer group was the result of moving away from the area, lack of availability of the volunteer for sampling, or knowledge that the volunteer was not drinking treated water. No volunteer had to be dropped because of illness or medical complaints.

All the Abate used to prepare the granules was identical to that employed in the study reported earlier (Laws et al., 1967).

The scheduling of the various procedures in the field study was as follows.

Insectical treatment of the area

All drums, barrels, and cisterns containing potable water were treated with a formulation of 1%

Abate adsorbed on sand, which, if all the Abate were released into 50 US gallon. '88 litres) of water, would produce a concentration o.' 1 ppm of Abate (i.e., 18.9 g of sand granules per drum). This treatment was repeated at monthly intervals from December 1965 through July 1967 except that the 13th monthly treatment in January 1967 was omitted. The rate of addition of Abate was determined by the size of the container; no account was taken of the amount of water which happened to be present at the time of each treatment. In addition, all premises were sprayed and all containers of non-potable water in the study area were treated with 2.5% malathion emulsion at intervals of 2 months. The Cerro Gandía area was sprayed by airplane with technical-grade malathion at the rate of 3 US fluid ounces per acre (about 220 ml/ha) once only at the end of the first 6 months of the study (June 1966). It should be emphasized that no additional measures, such as clean-up and source reduction programmes or screening or covering of drums, were used to supplement control, and no systematic control—chemical or otherwise—was practised in the surrounding area.

Toxicological and medical surveillance

Samples of blood (5 ml–10 ml of whole blood obtained by venepuncture in heparinized tubes) and urine (100 ml or more) were obtained prior to beginning treatment and at intervals of 1, 2, and 4 weeks, 6 months, and 1 year after beginning the treatment schedule. Additional urine samples were obtained at monthly intervals 15 days after each Abate treatment. These samples of blood and urine were analysed at the Toxicology Laboratory, Pesticides Program, National Communicable Disease Center, Atlanta, Ga., USA.

The blood samples were analysed for red blood cell and plasma cholinesterase by automated potentiometric titration at a constant pH, a modification of the method of Jensen-Holm et al. (1959).

Urine samples were analysed for ether-extractable organic phosphorus, and the results were expressed as Abate-equivalent based on the molecular weight and phosphorus content of Abate (Mattson & Sedlak, 1960). The lower limit of sensitivity of the method was 0.05 ppm.

The medical staff and nursing units of the local public health unit and municipal hospital were advised of the study and alerted to report any cases of suspected organic phosphorus poisoning, any unusual incidence of any disease or condition, or any unusual disease or symptom complex occurring in the inhabitants of the study area. The local office of vital statistics was also contacted and it co-operated in this aspect. The health unit, municipal hospital, and office of vital statistics were visited at least once a month by one of the directors of the study. In addition, frequent inquiries were made among the participants in the study.

Chemical analysis of water

Samples of water were taken from 20 of the treated barrels 48 hours after treatment with Abate. These samples were analysed for concentration of Abate at the Tec'nical Development Laboratories of the National Communicable Disease Center, Savannah, Ga., USA.

Upon receipt of the water samples from the field, 300-ml aliquots were extracted three times with 25-ml portions of chloroform and the extracts were combined. After evaporation of the solvent, the Abate content was determined by oxidation of the residue with perchloric acid and colorimetric determination by the molybdenum-blue method or by hydrolysis of the Abate with methanolic KOH and measurement of the absorbance of the resulting 4,4′-thiodiphenol at 263 nm. In some cases, contaminants in the water interfere with the analyses, making a clean-up procedure necessary to obtain reproducible results. The most satisfactory clean-up procedure was to spot the total extract on a thin-layer plate of silica gel, develop the chromatogram with hexane-ether solution and cut out the Abate spot for quantitative determination.

Biological evaluation

The entire study area was inspected for *Aedes aegypti* infestation every 2 months. Both exterior and interior surveys were made. The index for each was based on the percentage of premises found infested.

RESULTS

Toxicological and medical surveillance

No significant change was observed in either plasma or red blood cell cholinesterase at any time during the study; the results of all determinations were within the normal limits.

The results of the analysis of urine are given in Fig. 1. It appears that equilibrium was reached after about 20 weeks of dosage. The Abate-derived compound in the urine dropped rapidly to near control

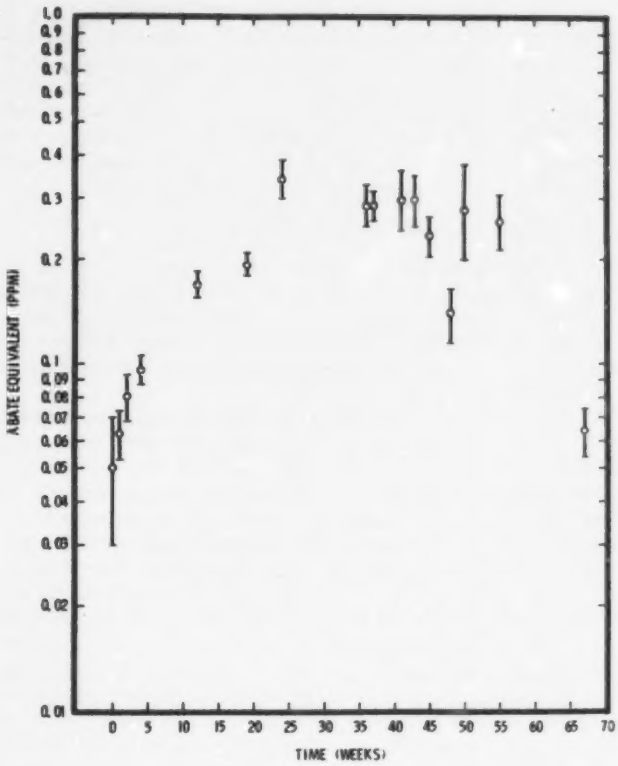


FIG. 1
URINARY CONCENTRATION OF ABATE EQUIVALENT (ppm) IN PEOPLE WHOSE WATER SUPPLY WAS TREATED WITH THIS COMPOUND [a]

[a] The points represent means and the bars represent 1 standard error.

levels after the newly installed water supply provided untreated drinking-water to most of the volunteers. An apparently significant drop in the equilibrium urine levels occurred in early November 1966. This is probably explained by the documented heavy rainfall (4–5 inches, or about 100 mm–130 mm, above normal) in late October, which was enough to reduce the concentration of Abate in the containers.

Because the municipal water supply was changed in March 1967, very few of the original volunteers continued to drink water treated with Abate. Therefore values for urine samples taken after April 1967 have little reliability or scientific value, and they are not reported. It should be noted, however, that the people continued to store water in barrels that continued to be treated with Abate. Thus, data concerning chemical analysis of water samples and infestation of containers by larvae remained valid, and medical surveillance was continued throughout the period in which any water was treated with Abate.

At no time during the study were there any serious illnesses or medical complaints among the volunteer group. Eight babies were born to members of the volunteer group, and all were normal. Four of these babies were conceived and delivered during the Abate study.

Monthly checks with the local public health unit, municipal hospital, and office of vital statistics revealed no change in the birth or death rate, number of spontaneous abortions, or number of stillbirths. No known congenital abnormalities occurred during the study, nor were there any unusual illnesses or deaths among the residents of Cerro Gandía. At least 42 children were born in Cerro Gandía during the study, and at least 22 were conceived after treatment with Abate had begun. The pregnancies and deliveries were normal; the babies were normal; and no neonatal deaths were recorded.

One stillbirth involving a woman who lived just outside the study area occurred in August 1966. The mother, a 24-year-old woman, had had two normal deliveries followed by two spontaneous abortions prior to the study. She lived at the foot of the hill directly opposite an operable public water faucet and denied ever drinking Abate-treated water.

At scattered times during 16 months of treatment, 32 individuals out of the population of about 2000 refused Abate treatment of their water drums. The reasons given for refusal are summarized in Table 2. All but 6 of these people refused only

TABLE 2

REASONS GIVEN FOR REFUSAL[a] OF ABATE TREATMENT OF DRUMS DURING 16 MONTHS OF TREATMENT (200 PREMISES)

Reason given	No.
Allergy	5
Asthma	1
Stomach trouble	10
Diarrhoea	2
Bad taste and/or odour	8
No reason	6
Total refusals	32

[a] All but 6 are refusals of 1 or 2 treatments only out of the total of 16 treatments.

1 or 2 of the 16 treatments. The 4 people who refused more than 3 treatments were interviewed at the end of the study. One was a 47-year-old woman who had had asthma and multiple food allergies since childhood; she was able to link drinking Abate-treated water with fatigue only, and not with symptoms of either asthma or allergy. Another woman said she had stomach cramps once after drinking the water, but she refused all further treatments. A 38-year-old woman developed a pruritic confluent rash on her upper arms and torso after treatments had been in progress for 6 months. However, the rash still persisted 6 months after she refused further Abate treatment of her water drums. Another woman gave diarrhoea as her reason for refusing treatment of the water, but was unable to relate any convincing description of illness when questioned.

All complaints of bad odour of the water were checked by inspectors, but none was confirmed.

There were no complaints of unusual illnesses or deaths among livestock, which included chickens, dogs, pigs, and goats.

Chemical analysis of water

The results of analysis of water samples taken during the test period are summarized in Table 3.

TABLE 3

ABATE CONCENTRATIONS FOUND IN SAMPLES TAKEN IN MANATÍ TEST AREA

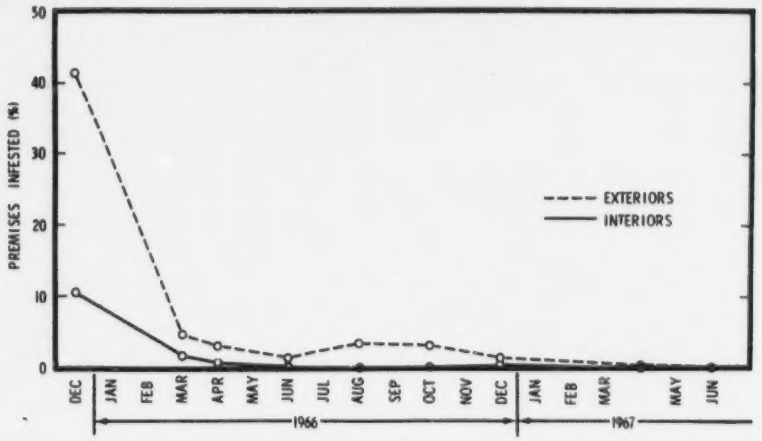
Month of treatment	Abate concentration found (ppm)		Method of analysis [a]
	Highest value	Average value	
1 January 1966	0.18	0.03	P
2 February 1966	0.17	0.02	P
3 March 1966	0.12	0.01	UV
6 June 1966	0.74	0.19	UV
10 October 1966	0.05	0.01	P-C
11 November 1966	0.05	0.02	P-C
12 December 1966	0.17	0.06	P-C
15 March 1967	0.46	0.11	P-C
16 April 1967	0.13	0.04	P-C
17 May 1967	0.15	0.06	P-C
18 June 1967	0.25	0.08	P-C
19 July 1967	0.03	0.02	P-C

[a] P = microphosphorus; UV = ultraviolet; P-C = microphosphorus after clean-up on thin-layer plates.

Although considerable variation was observed within each set of samples as well as from one month to another, the data clearly show that no excessive level was reached and no progressive build-up of Abate in the water reservoirs occurred even though the treatments were repeated for 19 consecutive months, with the exception of the 13th month. The data indicate that the water concentrations reflected the amount of rainfall, with higher concentrations appearing during dry periods. In no case was the nominal treatment concentration, 1.0 ppm, observed in any water sample. In fact, only one water sample was found to contain more than 0.5 ppm during the test period.

FIG. 2

PERCENTAGE OF PREMISES POSITIVE FOR LARVAE OF *AEDES AEGYPTI* IN THE AREA TREATED WITH ABATE



Biological evaluation

The index of *Aedes aegypti* infestation throughout the study is given in Fig. 2. It should be emphasized that Cerro Gandía is a geographically distinct area in the midst of a large town which did not receive any planned insecticidal treatment. Reinfestation of Cerro Gandía by *Aedes aegypti* was constantly possible. In spite of this, the exterior index in Cerro Gandía remained quite low; the interior index remained essentially at zero after the first week of treatment; and the over-all infestation index reached zero after 18 months of treatment.

Control was gradually achieved during the first 4 months of treatment. Minor reinfestation of some of the approximately 217 Abate-treated drums and barrels did occur after that period, but never in more than 3 drums during any given inspection cycle. Undoubtedly, some drums were missed during treatment. Occasionally, people acquired new drums, not previously treated, which might serve as a source of reinfestation until treated with Abate.

RÉSUMÉ

Au cours d'un essai pratique d'une durée de 19 mois organisé dans une collectivité de 2000 personnes à Porto Rico, de l'Abate adsorbé sur sable, à la concentration de 1 partie par million, a été ajouté à l'eau de boisson contenue dans les citernes et les réservoirs. Les applications ont été répétées chaque mois de décembre 1965 à juillet 1967. En outre, tous les locaux et les réservoirs d'eau non potable ont été traités par pulvérisations de malathion en émulsion à 2,5% à intervalles de deux mois et on a effectué un épandage aérien unique de ce même insecticide. Des analyses régulières d'échantillons de sang et d'urine prélevés sur des volontaires ont été effectuées en vue de rechercher les effets toxiques éventuels sur la population.

Aucun cas de maladie ou d'effets secondaires pouvant être attribués à l'emploi de l'Abate ou du malathion n'a été observé. On n'a pas noté de modification appréciable du taux de la cholinestérase sanguine, et les dosages du phosphore organique dans l'urine ont montré qu'un équilibre était atteint après 20 semaines environ d'applications. Trente-deux personnes seulement ont refusé à l'un ou l'autre moment de faire usage de l'eau traitée par l'Abate, les raisons invoquées n'étant guère convaincantes; le reste de la population a accepté facilement de la consommer. Les analyses chimiques de l'eau n'ont décelé ni teneur excessive en Abate ni accumulation progressive de l'insecticide en dépit de la répétition des applications. La concentration nominale initiale de 1 partie par million

n'a été notée dans aucun échantillon; un seul d'entre eux contenait plus de 0,5 partie par million d'insecticide.

L'élimination du vecteur *Aedes aegypti* a été obtenue progressivement durant les quatre premiers mois de traitement et s'est maintenue malgré la présence à la périphérie de la zone traitée de gîtes larvaires en activité.

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Pesticide Pollution In Soil And Water: The Need for Treatment of Wastewaters Containing Pesticides

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INTRODUCTION:

Today we are making great strides in learning to live in our environment in such a way that we also preserve it for the use of others. We are learning to appreciate our natural resources, recognizing that those who have the greatest potential for changing them also have the greatest responsibility for preserving them for both present and future use. A graphic example of the progress we are making is seen in the soil conservation programs initiated in the 1930's. In the State of Georgia, the countryside was characterized by huge erosion gullies and barren fields which had long since lost all traces of topsoil. Today very few of these erosion ditches are active, and a barren field is hard to find.

Similar strides are being made in protecting our soil and water from the less obvious, but equally damaging, effects of contamination and pollution, including even the invisible small quantities of toxic pesticides.

Need for Innovations in the Treatment and Control of Pesticide Residues

Forward looking industries now monitor their wastes very closely to make sure they maintain a high quality of discharge, and to signal corrective steps if the quality of their discharge deteriorates. New pesticides and formulations are being developed and recommended for use in ways that will confine their toxic effects to the proper time, place, and target.

To continue this progress toward a better environment a cooperative effort must be developed among all agencies, industries, and others concerned with the manufacture, formulation, use, and control of pesticides. We of the Research and Development arm of the Federal Water Pollution Control Administration are vitally interested in the development and application of new technology, innovations if you will, in terms of the theme of this conference, that will better protect our water resources. The Treatment and Control Activity at the Southeast Water Laboratory in Athens, Georgia, is particularly concerned with promoting research to resolve the problems encountered by the pesticide industry in facing up to its responsibility in the area of water pollution control.

Problems Caused by the Chlorinated Hydrocarbon Insecticides

Consider for a moment the chlorinated hydrocarbon group of insecticides. Endrin, the most toxic of these compounds to vertebrates, is acutely toxic to many fish at about one part per billion or less (1,2). Endrin has been responsible for many spectacular fish kills: spectacular both in numbers of fish killed (3) and the violent death fish suffer. Newspapers reporting a 1963 fish kill attributed to used sacks of endrin discarded in an irrigation canal near Sacramento, California, carried headlines about "bursting fish" that leaped out of the water and "exploded". Weiss reported (4) that "young guppies, which died due to protrusion of the brain through a bursting skull, may have exhibited an effect of endrin on the central nervous system that resulted in unusual pressure." Apparently, membrane permeability is drastically altered by endrin and related compounds.

DDT is practically ubiquitous throughout the world (5). It has been found in the oil of ocean fish in concentrations measuring 1 to 300 ppm (6); it has been found in concentrations up to 11 ppm in the eggs of the Bermuda petrel, a bird that lives over the open ocean and visits land only on a few rocky islets off Bermuda for breeding (6). DDT and other chlorinated hydrocarbon pesticides have even been found in rainwater in both the United States (7) and England (8).

Dieldrin, the most resistant to degradation of all organic pesticides, was the one found most frequently in a 1964 survey of major U. S. river basins (9). Endrin and DDT were second and third in frequency and detection.

Toxaphene was used in a cumulative dose of 50 ppb in Clayton Lake, New Mexico, to kill trash fish prior to stocking with rainbow trout (10). However, as late as nine months after treatment, the water still contained 1 ppb of toxaphene, and rainbow trout placed in the lake in a live-box were killed. Successful stocking was not accomplished until a year after the toxaphene treatment.

While water concentrations of these insecticides are typically found only in parts per trillion, there is real reason for the conservationists' concern that desirable species may be destroyed, eliminated, or impaired as a result of the effects of these pesticide residues in the environment. These very insoluble chlorinated hydrocarbon insecticides can be greatly concentrated in aquatic organisms both through the food chain and through direct absorption from the water.

While adult forms may not be directly affected, sublethal concentrations of pesticides have been reported to affect the reproduction of various species (11). A great deal of research was required to prove that female lake trout in Lake George, New York, concentrated enough DDT in their eggs to kill the fry after absorption of the yolk sac by the fry.

In Georgia and other states there is a growing awareness that the coastal estuaries are an essential breeding ground for many forms of aquatic life. Shrimp and oysters, the basis for sizable industries, are very susceptible in their larval stages (12,13) to pesticides toxicity. Since permissible tolerance concentrations of these pesticides in these species are not yet known, the effort is to keep all persistent pesticides out of the estuaries.

A great deal of work has been done to measure the persistence of pesticides, particularly the chlorinated hydrocarbons, in soils. Table 1 shows the range of persistence of some chlorinated hydrocarbons (14). Loss is greatest immediately after application, primarily because volatilization is very rapid until the pesticides become tightly sorbed to the soils. Even increasing humidity will displace a certain amount of chlorinated hydrocarbons from soil absorption sites (15). Table 2 is a summary of the relative persistence of chlorinated hydrocarbon pesticides as reported in several literature references (16-31). (The comparisons with arsenic and with the four thiophosphates were included in the same references.)

As would be expected, the chlorinated hydrocarbons are less persistent than arsenic, but much more persistent than the thiophosphates. Remember, though, that soil persistence is strongly affected by volatilization as well as by degradation. Dieldrin, for example, is not ranked as the most persistent chlorinated hydrocarbon, probably because it volatilizes more readily than the more persistent ones.

Table 1
APPROXIMATE RATES OF LOSS OF INSECTICIDES FROM ENGLISH SOILS
After Wheatley (14)

	Surface Application		Mixed into Soil	
Insecticide	50% loss (weeks)	90% loss (weeks)	50% loss (weeks)	90% loss (YEARS)
Lindane	4-6	30-40	15-20	2-3
Aldrin	1-2 +2% dieldrin*	5-6 +3% dieldrin*	10-15 +6% dieldrin*	1½-2 +25% dieldrin*
Dieldrin	8-10	(50-80)	210-360	(20-30)
p,p'-DDT	16-20	(80-100)	260-420	(25-40)

Figures in parenthesis obtained by extrapolation.
*Dieldrin formed by oxidation of aldrin.

Table 2
RELATIVE PERSISTENCE OF CHLORINATED HYDROCARBON AND OTHER PESTICIDES IN SOILS

Comparison	Reference
arsenic > DDT > BHC > chloridane > parathion	(16)
arsenic > DDT > BHC > parathion > chlordane	(17)
DDT > dieldrin > toxaphene > BHC	(18)
DDT > BHC > chlordane	(19)
DDT > BHC >>> guthion and malathion	(20)
DDT > aldrin + dieldrin > lindane	(21)
DDT > lindane > aldrin	(22)
DDT > BHC	(23)
toxaphene ≅ DDT >> BHC	(24)
endrin > DDT ≅ DDD > lindane	(25)
o,pDDT > p,p'-DDT	(26)
toxaphene > dieldrin > aldrin > chlordane >DDT > BHC > EPN = parathion (organic solvent extraction + chemical analysis)	(27)
dieldrin > aldrin > chlordane > DDT > toxaphene > BHC > EPN = parathion (organic solvent extraction + bioassay)	(27)
heptachlor > aldrin BUT aldrin + dieldrin > heptachlor + heptachlor epoxide	(28)
lindane > chlordane	(29)
BHC > dieldrin > DDT > chlordane > parathion bioassay with no extraction: test organisms exposed directly to the soil)	(30)
DDT > toxaphene > endrin > dieldrin > dilan >> chlordane > heptachlor > aldrin = isodrin > lindane > BHC	(31)

WEIGHTED SUMMARY: (NOT TRUE FOR ALL SOILS) more emphasis placed on those references citing more numerous tests.

arsenic >>>
o,p'-DDT > p,p'-DDT ≅ DDD > toxaphene >> endrin > dieldrin > dilan >>>
chlordane > aldrin ≅ isodrin > heptachlor > lindane > BHC >>
EPN, parathion, guthion, malathion

Problems Caused by Other Pesticides

The organic thiophosphate insecticides are not as persistent in soil and water as the chlorinated hydrocarbons, but they too have caused a great many fish kills. They have also been known to persist under peculiar circumstances for quite some time in the environment. Nicholson et al. (32) and Grzenda et al. (33) found 1.9 ppm of parathion in the bottom mud of a farm pond following a period of accelerated erosion nine months after parathion was applied to an adjacent orchard. These thiophosphates are chemically very similar to nerve gases and are highly toxic to most life forms having well-developed nervous systems. However, the toxicity of the thiophosphates to aquatic vertebrates is usually lower than that of the hydrocarbons.

Herbicides are a somewhat lesser problem in water pollution, yet it is very difficult to establish permissible tolerance limits for their use on aquatic nuisance plants. The contamination of ground water by herbicides at the Rocky Mountain Arsenal created severe problems because farmers of the area depended on that ground water to irrigate their crops (34, 35).

Treatment of Pesticide Laden Effluents

Effective treatment of wastewater from pesticide manufacture is extremely difficult. Removal of the thiophosphate insecticide residuals from such wastewaters can usually be accomplished by biological treatment or even chemical means, but adequate holding facilities must be available if a breakdown of treatment processes occurs. Fish kills associated with the manufacture of these pesticides have usually been caused by a simple bypass of the toxic wastes around the treatment plant, by a spill, or by some other failure to provide treatment.

Adequate treatment of manufacturing wastes containing chlorinated hydrocarbon insecticides appears extremely difficult. The most effective method of disposal appears to be deep well injection, although treatment of DDT wastes by alkaline hydrolysis is being attempted in at least one instance. Hill and McCarty (36) have shown that most of the chlorinated hydrocarbon pesticides degrade, or at least change to other forms, under anaerobic conditions. The Southeast Water Laboratory is initiating additional work along this line in an attempt to develop a waste treatment process that will be effective in degrading

at least some of the chlorinated hydrocarbons.

A tremendous amount of research and many published articles have dealt with pesticides and their effects on the environment, yet relatively little has been documented about techniques and processes for treating wastewaters containing these pesticides. The literature is particularly lacking in treatises regarding treatment of clorinated hydrocarbon insecticides. We of the Treatment and Control Activity of the Southeast Water Laboratory are attempting to document the experiences of various pesticide manufacturers in the treatment or control of their waste effluents. We feel that meaningful information can be obtained initially by simply cataloging that which is known, though not generally divulged by the industry. It is expected that this information, when pooled, will have value for all pesticide manufacturers without necessarily revealing process trade secrets. Of course, we would release no proprietary information without prior clearance from the firm concerned. If you or your companies have had particular experiences, successful or otherwise in the treatment of pesticide process wastewaters, we are anxious to discuss these with you.

Summary

It is recognized that pesticides are vital for maintaining our way of life. Mass production in agriculture, the production of good quality foods, and safeguarding the public health all require their use. We cannot, nor would we want to, simply turn back the clock and do away with pesticides; we must continue to manufacture, formulate, and distribute them. It is only reasonable, however, that we be increasingly vigilant to ensure that minimal harm is done to our environment through the manufacture and use of pesticides. Progress has been made in this direction; by enlisting the support of all concerned with the use of pesticides and the treatment of wastes from their manufacture of handling, still greater progress will be achieved.

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SURVEY OF INSECTICIDE SPRAY PRACTICES USED IN THE FRUIT ORCHARDS OF NORTH CENTRAL WASHINGTON

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WENATCHEE, WASH.

A SURVEY was made of the spray practices used to apply D. D. T. and parathion in the orchards of north central Washington. These data were needed as background material concerning the exposure of workers and concerning the amount of residue on fruits grown in orchards in which spray practices are carried out.

The survey was made during 1951 and 1952 and was based on data from 806 individual operations chosen at random from approximately 6,800 operations used to control insects on fruit trees in the Wenatchee and Okanogan Valleys. These valleys yield approximately half of the apples grown in the state of Washington. A large majority of the 806 operations on which the survey was based were carried out in accordance with recommendations published in 1951 and 1952 [1] by the Agricultural Experiment Stations of the State College of Washington. In general, both D. D. T.[2] and parathion [3] are applied in a single finished formulation. The rate of distribution per single application and the rate per season were studied.

The data have been tabulated with respect to the type and concentration of the formulation and the rate and method of application for the two major insecticides—D. D. T. and parathion. Table 1 gives the high, low, and mean of the rate per acre per application of insecticide [4] when applied as a constantly agitated water suspension [5] or as an emulsion to 237 orchards by stationary power-driven equipment,[6] to 318 orchards by truck- or tractor-drawn, power-driven equipment, and to 61 orchards by airplanes. The concentration and the rate of application of the formulations are also included as auxiliary information.

Table 2 gives the high, low, and mean of the rate per acre per application of insecticide when applied as a dust to 172 orchards by aircraft, including airplanes and helicopters, and to 18 orchards by truck- or tractor-drawn, power-driven equipment.

From the Communicable Disease Center, Public Health Service, United States Department of Health, Education, and Welfare, Atlanta.

1. Spray Recommendations for Tree Fruits in Eastern Washington, Extension Bulletin 419, State College of Washington, Agricultural Experiment Stations, Pullman, Wash., March, 1952.

2. 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane.

3. O,O-diethyl O-*p*-nitrophenyl thiophosphate.

4. It is not practicable to give the rate per tree, since there is little consistency in the number of trees per acre.

5. The term water suspension is used to cover those liquid formulations prepared by suspending water-wettable powders in an aqueous medium.

6. Locally spoken of as hand-spray equipment.

From a toxicological standpoint, the most important revelations in these tables are that D. D. T. was applied in the fruit orchards of north central Washington at rates of 1.0 to 25.0 lb. per acre per application and that parathion was applied at rates of 0.2 to 9.8 lb. per acre per application. The average rate per acre per application was 10.1 lb. for D. D. T. and 1.8 lb. for parathion. The rate of application per acre per season was approximately 20.3 lb. of D. D. T. and 3.7 lb. of parathion. Two applications are made on an average per season, and these usually occur in May and June. A slight variation occurred during 1952, since some growers used three applications of parathion due to the shortage of lime sulfur

TABLE 1.—*Summary of the Rates and Concentrations of D. D. T. and Parathion Water Suspensions and Emulsions Applied to Fruit Orchards in North Central Washington**

Method of Application	Values	Spray Gal./ Acre	D. D. T.			Parathion		
			Lb./100 Gal. Spray	Per Cent Formulation W/V	Lb./Acre/ Application	Lb./100 Gal. Spray	Per Cent Formulation W/V	Lb./Acre/ Application
Stationary power-driven equipment (237 applications)	Highest	3,200	1.5	0.18	22.5	2.0	0.24	5.0
	Lowest	100	< 0.1	< 0.01	1.0	0.01	0.001	0.2
	Mean	1,400	0.9	0.10	15.1	0.2	0.03	2.8
Portable power-driven equipment (318 applications)	Highest	1,800	50.0	6.00	25.0	6.0	0.72	9.8
	Lowest	32	0.5	0.06	1.5	0.1	0.01	0.2
	Mean	450	6.8	0.93	9.4	1.1	0.13	1.6
Aircraft (61 applications)	Highest	20	50.0	6.00	10.0	25.0	3.00	5.0
	Lowest	20	10.0	1.20	2.0	5.0	0.60	1.0
	Mean	20	30.9	3.71	7.0	12.0	1.44	2.5

* These values are based on the amount applied per acre per application. (The large majority of the orchards receive at least two insecticide applications during the growing season.)

TABLE 2.—*Summary of the Rates and Concentrations of D. D. T. and Parathion Dust Applied to Fruit Orchards in North Central Washington**

Method of Application	Values	Dust Lb./Acre	D. D. T.		Parathion	
			Per Cent Formulation W/W	Lb./Acre/ Application	Per Cent Formulation W/W	Lb./Acre/ Application
Aircraft (172 applications)	Highest	50	25.00	12.5	4.00	2.0
	Lowest	50	20.00	10.0	2.00	1.0
	Mean	50	23.96	12.0	2.80	1.3
Portable power-driven equipment (18 applications)	Highest	60	20.00	12.0	2.88	1.6
	Lowest	60	17.86	10.7	2.00	1.2
	Mean	60	18.30	10.3	2.40	1.4

* These values are based on the amount applied per acre per application. (The large majority of the orchards receive at least two insecticide applications during the growing season.)

which is usually used as a dormant spray. When D. D. T. and parathion were applied in a single formulation, the ratio of D. D. T. to parathion was always greater than 1, being about 2 to 1 in favor of D. D. T. in the case of finished liquid formulations and about 10 to 1 in favor of D. D. T. in the case of dusts.

Considering all methods of application, the liquid formulations, including water suspensions and emulsions, were used about three times more frequently than were dust formulations. In the early part of the growing season the ratio was even higher in favor of the liquid formulations, while in the latter part of the season the ratio was approximately 1 to 1.

The finished liquid formulations were much less concentrated than were the corresponding dust formulations. However, the liquid formulations were applied at a

much higher rate per acre. Thus, the rate of application of insecticide was approximately the same from both types of formulations. In estimating operator exposure and possible residue, the difference in the concentrations of these two types of formulations should be taken into account, along with the physical difference between the liquid formulation and the dry solid dust.

It should be pointed out that 90% of the finished liquid formulations were applied by ground equipment and that about the same proportion of dust formulations were applied by aircraft.

While this report is confined to the major insecticides used in the area—D. D. T. and parathion, consideration should be given to the fact that other insecticides, mainly tetraethyl pyrophosphate, Aramite,[7] malathion,[8] and methyl parathion,[9] as well as fungicides, for example, sulfur compounds, and plant growth regulators, for example, α-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid (2,4-D), are being applied to the orchards of north central Washington.

In a separate study now under way, a number of air samples have been collected in areas receiving applications of D.. D. T. and parathion. The latter studies, especially as they related to hazards to human beings, will be reported in a separate publication.

7. Manufactured by Chemical Division of the United States Rubber Co., Naugatuck, Conn.
8. S-(1,2-dicarbethoxethyl)O,O-dimethyl dithiophosphate.
9. O,O-dimethyl O-*p*-nitrophenyl thiophosphate.

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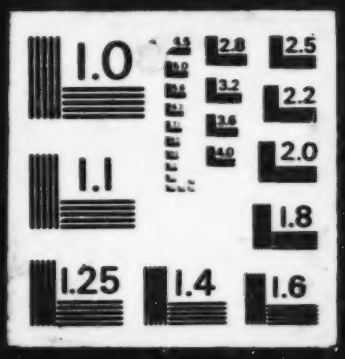

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FIELD STUDIES OF GARDONA AGAINST *AEDES AEGYPTI* IN PUERTO RICO

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In the chemical control operations of the Aedes aegypti Eradication Program in Puerto Rico during 1965 to 1968, the principal insecticide used for premises treatment was malathion, since the species was resistant to both DDT and dieldrin (Flynn *et al.*, 1964). The chemical was dispensed by hand and/or power equipment as a 2.5 percent emulsion which was applied to all containers holding nonpotable water, such as bottles, tires and tin cans; treatment was made on a block by block basis. Since laboratory and simulated field tests had indicated other organophosphorus compounds to be superior to malathion, such chemicals were field-tested on a small scale in Puerto Rico and at Perrine, Florida. In Florida (Eliason and von Windeguth, unpublished manuscript) three compounds, Abate,[6] Gardona and bromophos, were shown to be superior to malathion as a larvicide against *Aedes aegypti* breeding in small containers. Limited tests with bromophos and malathion in two municipios in Puerto Rico also suggested that malathion was less effective. In view of these promising results, emphasis was placed on conducting a large-scale evaluation of one of the three above mentioned compounds in two municipios in Puerto Rico. Since the biological efficacy of the three compounds against *Ae. aegypti* was similar, selection of the test insecticide was based on market availability, registration, mammalian hazard, and cost of each compound. On the basis of these criteria, Gardona was chosen as the test insecticide for the 1968 studies reported in this paper.

MATERIALS AND METHODS

(a) STUDY AREAS. Three municipios, Corozal, Naranjito and Morovis, 15 to 20 miles west of San Juan, Puerto Rico, none of which had a history of previous treatment by the Aedes aegypti Eradication Program, were selected as experimental areas (Figure 1). Corozal consisted of one urban community (1,340 premises) and 12 rural barrios (625 premises). Naranjito had a single urban community (800 premises) and seven rural barrios (776 premises). Morovis, where only the urban area of 750 premises was involved, served as an untreated town in the first round of spraying and as a treated community in the second spray cycle.

(b) CONTROL PROCEDURES. All urban and rural premises in Corozal and Naranjito were treated with Gardona suspension. In the first spray cycle[7] a 1.25 percent strength was used in the former, 2.5 percent in the latter. In the second spray

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[5] Present address: 4203 La Plata Avenue, Apartment "L," Baltimore, Maryland 21211.

[6] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

[7] The first spray cycles for urban Corozal and Naranjito were April 1 to May 13 and April 15 to May 9, respectively. The rural evaluative areas in Corozal and Naranjito were treated May 9 to 13 and May 10 to 28, respectively.

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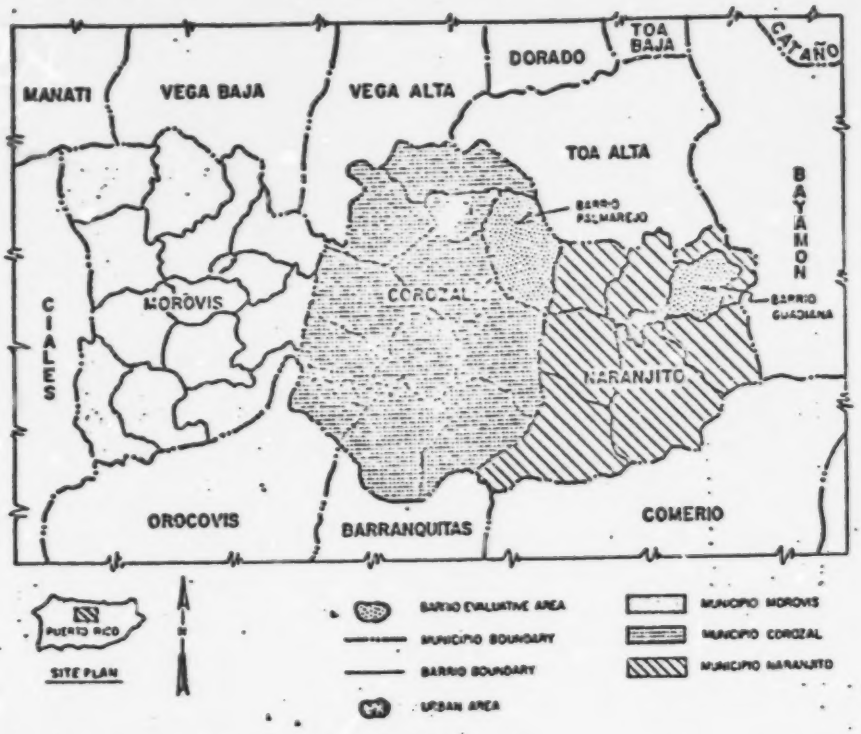

FIG. 1.—Map of areas.

cycle [8] both municipios were sprayed with 2.5 percent suspension. The required "charge" of Gardona was carried by each sprayman in individual plastic bags so that one charge in a hand sprayer can plus 2 gallons of water provided the correct concentration. Most of the premises were treated by this method, but power equipment was used for spraying trash dumps and open lots. Potable water and animal watering pans were dosed with 1 percent Abate sand granules, and dichlorvos resin strips were installed in the few cisterns involved.

Application procedures followed those normally employed in control operations in Puerto Rico except that no insecticides were used in the interior of houses and one individual of each two-man spray crew marked the containers with yellow paint before spraying them. This marking was necessary to enable the evaluation inspector to be certain that the container inspected had been treated, a procedure unnecessary in regular spray operations. In addition, spraymen frequently agitated their spray cans to prevent settling of the suspension, a procedure not followed with the emulsion formulation used on the operational program. Because of the large area involved in the rural portion of each municipio, one barrio in each was selected to represent the remaining rural zone. Barrio Palmarejo (87 premises) in Corozal and Barrio Guadiana (93 premises) in Naranjito were chosen. The former represented 14 percent of the total rural premises, the latter, 12 percent. These barrios

[8] The second spray cycle for Corozal was August 28–September 21 and for Naranjito, September 19–28.

were treated the same week, and all inspections in the rural areas were conducted therein.

(c) ENTOMOLOGICAL EVALUATION. Survey teams inspected containers on the premises, open lots and dumps in the urban areas and recorded whether they were positive or negative for mosquito breeding. Except for tremendous numbers of containers at the dump sites, all containers were checked. Records were maintained to show whether the container found was marked (*i.e.*, painted yellow) or unmarked, wet or dry, and positive or negative for *Aedes* larvae (*aegypti* or *mediovittatus*). All containers reported as positive for *Ae. aegypti* were also inspected and confirmed by the supervisor.

Ovitraps (Fay and Eliason, 1966; Jakob and Bevier, 1969) also were used in the same areas where larval surveys were conducted. Distribution was on a 350-foot grid pattern in the urban areas and the same design was followed in rural areas where feasible. With either method pretreatment assessments were made. Posttreatment larval surveys were scheduled at monthly intervals, but in practice the periods occurred from 4 to 5 weeks apart. Ovitraps were inspected weekly.

(d) EVALUATION OF CHOLINESTERASE LEVELS. To detect possible effects of the Gardona application on the spraymen, blood samples were taken from an initial group of 30 persons. The number unavoidably decreased, and the ultimate group that was consistently checked over the first spray cycle consisted of 16 persons. The local health department collected blood samples by venal puncture from the spraymen at intervals ranging from 1 to 3 weeks. The blood samples taken in vacutainers containing sodium heparin were centrifuged, separated and refrigerated, and the cell fractions were shipped to Perrine, Florida, where cholinesterase determinations were performed by the Perrine Primate Laboratory. The pH stat method, as described by Nabb and Whitfield (1967), was used to determine the values.

RESULTS

FIRST SPRAY CYCLE

Municipio Corozal: In urban Corozal pretreatment surveys were conducted March 4–8 and March 18–22 with larval indices as shown in Table 1. In the 10,091 containers surveyed, the average percentage of containers positive for larvae of *Ae. aegypti* was 7.4. A total of 1,266 premises were sprayed with 1.25 percent Gardona suspension by using hand units and 215 by power equipment. Approximately 49,000 containers were sprayed. Two weeks previously, of 4,267 containers with water, 6.1 percent were positive for *Ae. aegypti* larvae. Five weeks after treatment the index for the treated ones was 0.08 percent; at 10 weeks it rose to 1.1. On the first posttreatment inspection 24,482 containers were dry, on the second, 19,112. The data indicate that 2,505 and 2,215 wet unmarked or untreated containers were detected on the first and second posttreatment surveys, respectively, of which 1.9 and 4.8 percent were positive for *Ae. aegypti* larvae. In comparison with the treated cans, the in-

TABLE 1.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Corozal, first spray cycle, of 1.25 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
−4	1258	20.6	....	....	6824	7.2	6824	7.2
−2	1283	11.6	....	....	4267	6.1	4267	6.1
Treatment[1]								
+5	1283	2.9	3704	0.08	2505	1.9	6209	0.8
+10	1283	8.3	2490	1.1	2215	4.8	4705	2.8

[1] April 1–11, 1968.

festation rates for the untreated wet containers were 23.7 and 4.3 times higher. In untreated Morovis (Table 2) the larval positive indices for the containers marked during inspection I were 19.1 and 19.7 percent. On inspections II and III unmarked containers in Morovis showed an average 16.0 and 17.5 percent to be positive for larvae.

In the rural area of Corozal, Barrio Palmarejo, 87 premises were hand sprayed; 17 received the treatment by power equipment. In this area the pretreatment index for larval positive containers was 1.9 percent (Table 3). At 5, 9, 13, 17 and 21 weeks after treatment the indices for wet treated containers were 0, 0, 0, 0.8 and 0 percent, respectively. For the same successive weeks the indices for wet untreated containers were 0, 1.1, 2.7, 2.2 and 2.1 percent.

The data from the 75 ovitraps dispersed in urban Corozal revealed that 10.6 to 17.8 percent were positive for eggs of *Ae. aegypti* during weeks 19 to 13. During the spraying period of week 14 through week 19 the weekly indices for positive ovitraps were 13.2, 15.8, 13.2 8.0, 6.6 and 9.2 percent. On week 20 the index dropped to 1.3 percent, but the following week it rose to 17.3 percent and remained at levels between 11.3 to 21.3 percent for the next 3 weeks. For the weeks 17 to 20 the percentage of positive traps remained below 10.

In Barrio Palmarejo pretreatment ovitrap indices for a 5-week period indicated that eggs were present in 3.1 to 18.8 percent of the 32 traps used. After treatment the ovitrap indices fluctuated from 0 to 9.7 percent, and none of the traps were positive on 4 of the 9 posttreatment weeks.

TABLE 2.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in untreated Morovis, first spray cycle.

Inspection No.	Premises No. Insp.	Premises % Pos.	Marked Containers No. Insp.	Marked Containers % Pos.	Unmarked Containers No. Insp.	Unmarked Containers % Pos.	All Containers No. Insp.	All Containers % Pos.
I [1]	712	25.1	....	....	2925	15.9	2925	15.9
II [2]	686	11.7	1825	19.1	868	16.0	2693	18.5
III [3]	710	22.0	1173	19.7	926	17.5	2099	18.7

[1] Inspection and marking of containers April 22–27, 1968.
[2] Inspection 5 weeks after [1].
[3] Inspection 10 weeks after [1].

TABLE 3.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in Barrio Palmarejo, Municipio Corozal, first spray cycle, of 1.25 percent Gardona suspension.

Inspection (weeks)	Premises No. Insp.	Premises % Pos.	Treated Containers No. Insp.	Treated Containers % Pos.	Untreated Containers No. Insp.	Untreated Containers % Pos.	All Containers No. Insp.	All Containers % Pos.
<1	87	10.3	..	..	776	1.9	776	1.9
Treatment [1]								
+5	86	0	77	0	63	0	140	0
+9	84	4.8	164	0	548	1.1	712	0.8
+13	83	6.0	105	0	182	2.7	287	1.7
+17	84	6.0	132	0.8	268	2.2	400	1.8
+21	84	7.1	130	0	329	2.1	459	1.5

[1] April 29–May 3, 1968.

In urban Corozal the ovitraps also were positive for eggs of *Ae. mediovittatus*, and indices for this species followed the same general trends as for *Ae. aegypti* except that they were slightly lower. In Barrio Palmarejo, however, the oviposition findings suggested that *Ae. mediovittatus* was much more prevalent, since indices of 40 percent or greater were recorded for 11 of the 15 survey weeks. The insecticide treatment thus appeared to have less effect on this species than on *Ae. aegypti*.

Municipio Naranjito: In urban Naranjito pretreatment surveys were made March 11 to 13 and March 25–26 (Table 4). The combined index for these two surveys in wet containers showed that 1.3 percent of the containers were positive for larvae of *Ae. aegypti*. Approximately 749 premises were then treated with hand sprayers; 78 with power units. The number of containers sprayed totalled 51,575. At the 5-week posttreatment inspection 1,157 containers were wet, but all were negative for *Ae. aegypti* larvae. The inspections at 9 and 13 weeks after spraying showed indices in the wet treated containers of 0 and 0.3 percent. Untreated wet containers showed indices of 0.1, 0.4 and 0.7 at the 5-, 9- and 13-week inspections, respectively. Indices for untreated Morovis are given in Table 2.

In Barrio Guadiana, the rural evaluative portion of this municipio, 93 premises were hand sprayed; 52 were treated with power equipment. The pretreatment index for the 262 wet containers was 6.8 percent (Table 5). Posttreatment inspections at 5, 9, 13, 17 and 21 weeks were negative except for two positive containers detected at week 21. Untreated wet containers in this area had indices ranging from 0.8 to 3.8 over the same time span.

Ovitrap indices for *Ae. aegypti* in urban Naranjito did not exceed 4.5 percent for any of the pre- and posttreatment weeks from 10 to 28, and no marked change occurred during the treatment period. In contrast, indices of 14 to 41 percent were recorded for *Ae. mediovittatus*, but the treatment exerted no apparent detrimental effect on oviposition. In Barrio Guadiana, the ovitrap index for *Ae. aegypti* eggs ranged from 0 to 6.4 percent, but the treatment apparently had little influence on oviposition. *Ae. mediovittatus* eggs were recovered from 17.0 to 45 percent of the 47 ovitraps during the 12-week observation period.

TABLE 4.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Naranjito, first spray cycle, with 2.5 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
−5	778	3.3	..	..	2085	1.5	2085	1.5
−3	765	2.5	..	..	1803	1.1	1803	1.1
Treatment [1]								
+5	770	0.1	1157	0	794	0.1	1951	0.05
+9	768	0.4	607	0	740	0.4	1347	0.2
+13	755	1.0	311	0.3	1171	0.7	1482	0.6

[1] April 15–19, 1968.

One of the obvious factors apparent in both municipios was the rapid turnover in containers. Data are given in Table 6 which demonstrate this point. In urban Corozal the inspection 5 weeks after treatment revealed that of 48,771 containers inspected 42 percent represented unmarked containers that were not present when the area was sprayed. On the survey at 10 weeks, 52 percent of the 45,151 containers were new. In Naranjito the same progressive increase in new or unmarked containers occurred, as was true for the rural areas of each municipio and for untreated Morovis.

During the first spray cycle (April 1 through May 28) a total of 8,153 gallons

TABLE 5.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in Barrio Guadiana, Municipio Naranjito, first spray cycle, with 2.5 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
<1	93	24.7	..	..	362	6.8	562	6.8
Treatment[1]								
+5	93	2.2	238	0	127	2.4	365	0.8
+9	91	3.3	259	0	400	0.8	659	0.5
+13	92	3.3	79	0	228	1.3	307	1.0
+17	93	11.8	103	0	437	2.7	540	2.2
+21	91	18.7	188	1.1	469	3.8	657	2.7

[1] April 29–May 3, 1968.

of Gardona was applied to the premises of the two municipios. One-third of this amount represented the 2.5 percent formulation, the balance 1.25 percent. In urban Corozal each two-man team applying the insecticide with hand sprayers averaged five premises per 5-hour day, whereas in urban Naranjito the average was 6.8 premises. Coverage of the rural premises was much slower, the average ranging between 2.0 and 2.2 premises. Treatment with power equipment involved 6,450 gallons of Gardona spray, with 62 percent being applied in urban Corozal.

The data for cholinesterase values of 10 spraymen, two supervisors, two packagers and controls are listed in Table 7. All values were considered to be in the normal range of variation except for those of sprayman number 10 and the two packagers on April 19. On April 24 these men were assigned to duties not exposing them to Gardona or other insecticides. However, samples from the same individuals on April 25 indicated normal values for each; consequently, the men returned to their regular duties and subsequently none of those individuals showed any abnormal values. Since the recovery to normal levels was more rapid than would normally be expected, it was concluded that the low readings obtained with the April 19 samples were probably due to accidental contamination of the sampling tubes with

TABLE 6.—Number of containers inspected in three municipios and percentage of marked and unmarked (untreated) containers detected on each survey.

Area	Weeks after Treatment	Total	Percent Marked	Percent Unmarked
Urban Corozal	5	48,771	58	42
	10	45,151	52	48
Rural Corozal	5	4,222	59	41
	10	4,101	40	60
Urban Naranjito	5	18,570	53	47
	9	19,046	34	66
	13	16,122	22	79
Rural Naranjito	5	6,505	57	43
	9	5,242	40	60
	13	5,751	28	72
Urban[1] Morovis	0	26,035	100	..
	5	21,596	65	35
	10	25,987	53	47

[1] Untreated town in which containers were marked at time of first inspection.

TABLE 7.—Cholinesterase levels[1] in persons spraying Gardona suspension (1.25 and 2.5%) packaging 75% Gardona wettable powder, and in supervising spraymen.

		Preexposure		Exposure				
		Mar. 8	Mar. 29	Apr. 5	Apr. 19	Apr. 25	May 10	May 24
Spraymen								
1	RBC	11.9	14.4	12.3	10.8	..	20.0	13.4
	Plasma	4.0	4.3	3.5	3.6	..	6.5	2.4
2	RBC	12.9	15.5	13.2	10.8	..	22.0	14.0
	Plasma	6.8	4.8	5.3	4.7	..	5.7	4.7
3	RBC	12.6	13.9	..	12.6	..	21.0	16.8
	Plasma	5.5	4.9	..	5.1	..	6.4	4.5
4	RBC	11.2	12.8	12.1	..	..	..	15.4
	Plasma	4.9	4.9	4.4	..	..	..	4.0
5	RBC	11.7	15.5	14.6	13.7	..	18.2	13.7
	Plasma	6.4	4.9	5.5	5.5	..	4.7	5.4
6	RBC	11.2	13.4	11.6	9.1	..	20.0	16.5
	Plasma	4.1	4.8	3.8	3.9	..	5.2	3.2
7	RBC	13.3	13.9	..	12.3	14.9	19.6	14.1
	Plasma	4.7	4.8	..	4.1	4.7	4.9	3.2
8	RBC	12.2	10.7	13.1	10.0	..	18.0	14.6
	Plasma	5.0	4.4	4.4	4.7	..	5.1	2.7
9	RBC	13.4	13.9	13.0	13.8	14.9	20.0	14.7
	Plasma	4.8	4.8	3.9	4.0	4.4	4.6	2.7
10	RBC	..	13.9	..	4.8[3]	17.6	19.4	14.3
	Plasma	..	4.1	..	3.3	3.7	5.3	3.0
Packagers								
1	RBC	..	16.6	12.4	5.4[3]	18.0	22.0	15.6
	Plasma	..	4.8	3.4	1.8	3.9	5.0	2.0
2	RBC	..	..	12.2[2]	7.0[2]	14.0	13.6	14.4
	Plasma	..	..	5.3[2]	3.8	5.6	3.8	3.8
Spray Supervisors								
1	RBC	13.0	14.4	11.7	13.2	..	17.5	13.6
	Plasma	4.7	4.8	4.4	4.6	..	6.2	3.6
2	RBC	15.0	15.0	13.0	16.4	..	22.0	15.6
	Plasma	5.3	4.9	4.1	4.6	..	5.0	2.0
Controls								
1	RBC	..	16.0	12.3	10.8	15.0	15.8	13.4
	Plasma	..	4.7	4.2	4.4	4.6	5.9	4.4
2	REC	..	..	12.2	13.6	15.9	18.0	13.4
	Plasma	..	..	5.3	5.3	6.2	6.7	5.2

[1] Values reported are expressed in micromoles acetylcholine hydrolyzed/minute/ml. plasma or packed red blood cells.

[2] Blood samples taken on April 15.

[3] These low values were considered probably to be due to accidental contamination of the sampling tubes with Gardona.

Gardona. The data for the personnel engaged in these operations with Gardona thus indicate that the insecticide can be safely applied as a premises treatment.

SECOND SPRAY CYCLE

From the results achieved in the first spray cycle, decision was made to re-treat urban Corozal and Naranjito with a 2.5 percent Gardona suspension, to continue inspection but not re-treat rural Corozal and Naranjito, and to spray Morovis with 2.5 percent Gardona suspension.

Municipio Corozal: During weeks 35 to 38, urban Corozal was re-treated using a 2.5 percent Gardona suspension with the same techniques followed in the first spray cycle. The index for containers positive for *Ae. aegypti* larvae at the time of treatment was 1.1 (see Table 1). At 4, 8 and 12 weeks after the second application, the indices in treated containers were 0.03, 0.03 and 0.3 percent (Table 8). In comparison, new untreated containers showed indices of 0.6, 0.6 and 1.8 percent for the same successive time intervals.

Ovitrap data for 75 units indicated approximately 30 percent positivity for each of the 3 weeks preceding treatment. The indices for positive ovitraps during the treatment period (weeks 35–38) were 37, 29, 21 and 20. For weeks 39 through 48 the indices ranged from 1.3 to 8.1.

Municipio Naranjito: Urban Naranjito was re-treated on weeks 38–39. The previous index of this community showed 0.3 percent of the containers to be positive for larvae of *Ae. aegypti* (Table 4). After re-treatment, indices of *Ae. aegypti* larvae ranged between 0 and 0.04 percent through week 17 (Table 9). On week 22 the index was 0.13 percent. In the unmarked containers the indices for weeks 4 and 8 were 0.08 and 0.09; for weeks 12 and 17, 0.25 and 0.35; and for week 22, 0.59 percent.

The percentages of the 45 ovitraps positive for eggs of *Ae. aegypti* for 3 weeks prior to treatment were 6.8, 4.4 and 4.4. On the first week of treatment (week 38) the index rose to 18.2 percent, but for the subsequent weeks 39 to 47 the maximum percentage of positive traps was 2.4.

Municipio Morovis: This untreated

TABLE 8.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Corozal, second spray cycle.

Inspection (weeks after treatment) [1]	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
4	1283	0.70	2770	0.03	1322	0.60	4092	0.21
8	1288	0.69	2642	0.03	1671	0.59	4313	0.25
12	1290	4.03	4971	0.30	3596	1.77	8567	0.92

[1] Re-treated August 28–September 21, 1968. Index before re-treatment was 1.1 (Table 1).

TABLE 9.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Naranjito, second spray cycle, with 2.5 percent Gardona suspension.

Inspection (weeks after treatment) [1]	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
4	785	0.12	2559	0	1237	0.08	3796	0.02
8	787	0.25	2332	0.04	1031	0.09	3363	0.05
12	770	0.51	2849	0	1545	0.25	4394	0.09
17	780	0.76	2118	0	1981	0.35	4099	0.17
22	794	0.37	1503	0.13	1505	0.59	3008	0.36

[1] Re-treated September 19–25, 1968. Index before re-treatment was 0.3 (Table 4).

community was sprayed during weeks 34 and 35. A survey on weeks 32–33 revealed that 18.3 percent of the 3,554 containers inspected were positive for *Ae. aegypti* larvae (Table 10). In inspections on weeks 4, 8, 12 and 16 after the application of Gardona, the indices in wet containers were 0.08, 0.11, 0.23, and 0.49. Unmarked containers showed indices in wet containers of 1.8, 2.1, 1.8 and 4.0 for the same successive intervals.

In the 4 weeks prior to treatment indices for ovitraps positive for *Ae. aegypti* eggs ranged between 43.2 and 47.2 percent. During the 2 weeks of treatment the indices were 48.6 and 16.7 percent. In the indices for the next 12 weeks, the percentage of ovitraps positive for eggs of *Ae. aegypti* ranged between 2.9 and 14.7, but no consistent trends were apparent.

TABLE 10.—Percent of premises and wet containers positive for *Aedes aegypti* in urban Morovis after treatment with 2.5 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
−2 Treatment [1]	830	28.91	..	..	3554	18.31	..	..
+4	815	3.68	3615	0.08	1818	1.76	5433	0.64
+8	824	5.09	3348	0.11	2194	2.09	5542	0.90
+12	835	4.79	2584	0.23	2355	1.78	4939	0.97
+16	817	4.03	2644	0.49	2336	3.98	4980	2.12

[1] August 19–30, 1968.

DISCUSSION

The data from these studies establish that Gardona applications of 1.25 or 2.5 percent suspensions provided marked reduction of larval indices of *Ae. aegypti* in treated containers in both rural and urban areas. In urban Corozal (2.5 percent suspension) the results of the inspection on the 10th week represented an 82 percent reduction when compared to the pretreatment indices. In relation to the index for the untreated containers, the reduction was 77 percent. Container indices for larval infestations in untreated Morovis were 19 times those of treated Corozal.

In urban Naranjito the percent reduction at week 13 was not as favorable as in Corozal, either in relation to original pretreatment index or to that for the untreated containers. The significance of these findings is lessened considerably because of the relatively low level of infestation (1.1–1.5 percent) before treatment. Nonetheless, the absence of any breeding for 9 weeks after treatment was encouraging, and the validity of this view is strengthened greatly when the data for Barrio Guadiana (Table 5) are reviewed. This area, with a pretreatment level equal to urban Corozal (6.8 percent) and much higher than that for urban Naranjito, showed a complete absence of treated containers positive for *Ae. aegypti* larvae on the inspection 17 weeks after it was sprayed with 2.5 percent suspension. In contrast, the untreated containers had indices of 0.8 to 2.7 percent. In Barrio Palmarejo sprayed with 1.25 percent Gardona suspension, few infested containers were found 21 weeks after treatment, but here the pretreatment level was only 1.9 percent.

Re-treatment of urban Corozal and Naranjito with 2.5 percent Gardona suspension at times when the indices were low from the first cycle treatment showed that the applications essentially eliminated further breeding in the treated containers for 8 and 17 weeks, respectively. Moreover, treatment of Morovis (the untreated urban area for the first spray cycle) with 2.5 percent Gardona at a time when the

index was 18.3 percent demonstrated the high level of effectiveness of the Gardona application when a 97 percent reduction was present 16 weeks after application. Untreated containers showed an infestation rate of about 4.0 percent, approximately eight times that of the treated receptacles.

The data for all applications indicate that Gardona was highly effective in controlling or preventing the breeding of *Ae. aegypti* larvae in containers. Although the 1.25 percent formulation performed particularly well in Barrio Palmarejo, the results in urban Corozal and Morovis suggest that the 2.5 percent concentration would be the choice for control operations. The absence of adverse responses in the cholinesterase levels of the spraymen and other personnel handling Gardona supports the view that its use does not offer any marked hazard to man. Since the acute oral LD-50 dosage for male and female rats is greater than 4,000 mg./kg., these results were not unexpected (Gaines, 1969).

The results also suggest that premises applications based on 2, 3 or 4 monthly intervals are not sufficiently productive to achieve the ultimate aim of the program, the eradication of the species. Table 6 clearly shows that the rate at which new untreated containers accumulate in a community is so rapid that the mosquito population has numerous untreated sites in which to oviposit. In treated urban Corozal and Naranjito, the percentages of unmarked containers positive for larvae were 7 to 12 percent higher than in untreated Morovis, on the 5-week survey. This fact suggests that the insecticide treatment possibly might repel the female, thereby causing her to seek an untreated site for oviposition. The data at week 10 show little difference in the percentage of unmarked positive cans in Corozal and Morovis, but a 19 percent difference exists between Morovis and Naranjito. These findings do not discount the possible factor of repellency of the treated receptacles, since the containers in Naranjito were sprayed with 2.5 percent Gardona and theoretically could be less attractive to ovipositing females than those in Corozal treated with the 1.25 percent formulation. Such repellency has been reported from studies with insecticidal treatment of houses where the residues repelled or deterred anopheline mosquitoes from entering. Since the degree of repellency may differ with the insecticide used, this factor may also vary in its importance. In the Florida studies (Eliason and von Windeguth, unpublished manuscript) the *Ae. aegypti* larval indices (18.7 and 29.6 percent) of the treated wet containers were greater than those of the untreated containers (*i.e.*, 12.3 and 19.2 percent) in the same premises during the 8th and 12th week posttreatment inspections. In contrast, the Gardona-treated areas showed indices in wet containers of 1.3 and 1.6 percent versus 16.9 and 16.5 percent for the same time intervals. Such findings might suggest lack of repellency to malathion, but they definitely show a lack of effectiveness in controlling the species.

One of the critical factors in *Ae. aegypti* control in small containers is the rapidity with which new receptacles appear on premises. Von Windeguth *et al.* (1960) reported that during a 3-month period, the total number of containers in an urban area of nine blocks remained essentially the same. However, in the various blocks the percent of marked containers removed during that period ranged from 43 to 68 and averaged 55 for the nine-block group. Over the same period 53 percent of the containers found were new and unsprayed.

This rapid turnover in containers offers a serious obstacle to successful eradication procedures. The concept of an insecticide that would remain effective for 2 to 5 months loses its significance when new untreated containers are available for the species to continue its propagation. In addition, the ovitrap data from these tests suggest that the adult female mosquitoes present during the time of treatment continue to lay eggs for their life-span which probably extends beyond the period of treatment. Because of this condition, the possibility of repellency of treated contain-

ers to the adult female, and the rapid appearance of untreated containers, the chances of drastically and immediately curbing *Ae. aegypti* populations by premises treatment alone decline greatly. A partial solution to this problem would be the use of adulticiding measures simultaneously with the premises application so that adults would not be present to deposit their eggs in the new untreated containers that appeared in the area. Such adulticiding applications might well be required at monthly or less frequent intervals to insure that no ovipositing females would be present.

The necessity for frequent chemical operations focuses the attention again on the fact that sanitational measures to curb the indiscriminate disposal of these containers on premises or to remove such receptacles might prove the most feasible and effective eradication measure. Since this type of procedure *per se* is frequently a difficult practice to enforce, the operational approach that appears to be most feasible would be to use a combination of sanitational and chemical measures that would be followed within a given area until the biological data indicated successful eradication. Once the latter is achieved, the control activities would advance to adjacent areas, and the original operational area would be placed under surveillance only.

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